Evaluation of antimicrobial activity of *Artocarpus altilis* on pathogenic microorganisms

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Abstract

The study was aimed to evaluate the antimicrobial, MIC and MBC/MFC activities of *Artocarpus altilis* (twigs). In vitro antimicrobial activity was evaluated against six pathogenic microorganisms (two gram positive strains: *Staphylococcus aureus* (ATCC-25923) and *Bacillus cereus* (ATCC-11778), the Gram negative strains *Escherichia coli* (ATCC- 35218) and *Pseudomonas aeruginosa* (ATCC -27853), and two fungal strains: *Candida albicans* (ATCC -10231) and *Cryptococcus neoformans* (ATCC- 90112)) using the disc diffusion method at 2mg/disc by indicating the presence of the clear inhibition zones around each disc compared with the positive control (Streptomycin and Nystatin), while the MIC and MBC/MFC were ranged from (250-1000µg/ml) assayed by using the 96 well plates. The dried twigs of *A. altilis* were extracted by using the Soxhlet apparatus. The extracts of hexane and DCM were observed a moderate antimicrobial activity at (14.6±0.2mm) against *Bacillus cereus*, whereas 11.8±0.3mm and 11.5±0.2mm values for *C. albicans* respectively. The least MIC of 250µg/ml was obtained by DCM extract against *S. aureus* for bacteria and *C. albicans* and *C. neoformans* for fungus respectively. The results suggested that *Artocarpus altilis* extracts have promising therapeutic potential against bacteria and fungi.

Key words: Antimicrobial, MIC, MBC/MFC,*Artocarpus altilis,*therapeutic potential

INTRODUCTION

The *Artocarpus* spesiec contain a variety of secondary metabolites under phenyl propanoids group including flavonoids and flavones (Nomura et al., 1998). There are more than 130 metabolites have been identified in *Artocarpus altilis* (bread fruit), whereas more than 70 compounds are derived from the phenylpropanoid pathway(Iwaoka et al., 1994). Many of the isolated compounds have been found to exhibit biological activity including inhibition of platelet aggregation, anti-bacterial, anti-fungal, inhibition of leukemia cells and as an anti-tumor agent. These data support the claim that the breadfruit tree may be an effective medicine with the potential to treat various of medical cases. In addition, *Artocarpus* can be used as traditional medicine, the flowers is used to cure toothache, while its root is used to stop bleeding (Kochummen, 1987; Heyne, 1987). In Malaysia the fruit is a source of commercial foods. The stems and roots of this plant have traditionally been used in Taiwan for the treatment of liver cirrhosis, hypertension and inflammation. In addition, the wood part has been valued as a source of commercial timber (Ersam et al., 2002). The uniqueness of the structure of secondary metabolites in *Artocarpus* produce a broad physiological effects, such as anti-bacteria (Khan et al., 2003), antiplatelets (Weng et al., 2006), anti-fungus (Jayasinghe et al., 2004), anti-malarial (Widyawaruyanti et al., 2007; Boonlaksiri et al., 2000) and cytotoxic (Ko et al., 2005; Syah et al., 2006). Humans, animals and plants are exposed to infections from microorganisms. They depend on antibiotics to combat infection pathways. However, microorganisms have developed resistance to almost all the available antibiotics. This situation demands serious efforts for the development of new and effective antibiotics (De Sbois et al., 2008). Extracts from plants and pure secondary metabolites are rich sources of antimicrobial agents. The mechanism of inhibition of growth of microorganisms by the phytochemicals is different from that of the conventional antibiotics (Bhattacharjee et al., 2010).

Experimental

Collection, preparation of plant materials: The plant (twig) was collected from Taman Pertanian Sultan HajiAhmad Shah, Kuantan, Pahang. The sample was prepared for extraction by washing off all dirt and soil residues. It was left to dry in the oven at 40^{o}C for 72h. After drying, powdered (700g) twig part of *Artocarpus altilis* wassequentially extracted with different solvents polarities, i.e *n*-hexane, dichloromethane and methanol using Soxhletapparatus according to Green, (2004). Each solvent was removed *in vacuo* by rotary evaporator at 60˚C to obtain theextracts. Then, the extract was stored at 4˚C until further analyses.

Source of Microorganisms

The microbial strains used in this study were bacteria and fungi which were responsible for causing some pathogenic in human. The strains were susceptible to most antibiotics that were tested in clinical laboratories. The Gram-positive bacteria included *S. aureus* (ATCC-25923) and *Bacillus cereus* (ATCC-11778), the Gram negative strains *Escherichia coli* (ATCC-35218) and *Pseudomonas aeruginosa* (ATCC-27853), and two fungi *Candida albicans* (ATCC-10231) and *Cryptococcus neoformans* (ATCC-90112). All microbial strains were purchased from theInstitute for Medical Research (I.M.R), Kuala Lumpur, Malaysia.

Antimicrobial activity

The antimicrobial activity of the hexane, DCM and methanol extracts of *A. altilis* was determined by using discdiffusion method at concentration of 2mg/disc, (Murray et al., 1999). Inoculums containing 10^8 CFU/ml of bacteria and 10^6 CFU/ml of fungal was spread on nutrient agar for bacteria and potato dextrose agar for fungi plates strains respectively, using a bent sterile glass rod in such a way as to ensure through coverage of the plates uniformly. Sterile filter paper discs, 6 mm in diameter were impregnated with 20µl from the stock solution (100 mg/ml) of three crude extracts (hexane, DCM and methanol). The sterile filter paper was placed with a sterile forceps on the surface of the inoculated plates. The plates were left on the bench undisturbed for few minutes then, incubated at 37°C for 24 hours with bacterial and at 32 °C for 48 hours for fungus strains. The positive control used throughout this studywas Streptomycin (10 µg/disc) for bacteria and Nystatin (100 µg/disc) for fungus, while the negative control was methanol (30 µl/disc). The diameter was determined using ruler after incubation. The antimicrobial activity was indicated by the presence of the clear inhibition zones around each disc (Murray et al., 1999). Analysis of results were done by measuring the inhibition zones in millimeter diameter and calculating the Standard Deviation (SD) from the triplicate measurements.

Determination of (MIC) and (MBC/MFC) of the crude extracts.

The MIC was determined for each crude plant extract inhibiting antimicrobial activity against test pathogenic microorganisms. MIC assay was performed by a broth micro dilution technique, using 96- well micro-liter plates according to Sarker et al., (2007). Plant extracts were prepared in MeOH to make 2000µg/ml as a final concentration. This concentration was then serially diluted by adding to the broth media in a 96-wells micro-liter plates to obtain 1000, 500, 250, 125, 62.5, 31.2 and 15.6µg/ml. Then, 100µl inoculum (10^8 CFU/ml for bacteria and 10^6 CFU/ml for fungi) was added to each well, while broth containing standard drug (Streptomycin and Nystatin) was used as a positive control. The micro-wells plates were incubated at 37ºC for 24 hours for bacteria and 32ºC for 48 hours for fungi. The turbidity of the micro-well plates was shown as visible growth of microorganisms. To determine the minimum bactericidal/fungicidal concentration (MBC/MFC) further sub culture was made unto agar plates as described by Vollekova et al., (2001) and Usman et al., (2007). In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate The lowest concentration of the extract which showed no visible growth on the agar was noted and recorded as the MBC/MFC.

Results

Table 1. showed that *A. altilis* twigs had a moderate antibacterial activity for hexane and DCM extracts with the same zone of inhibition value of 14.6±0.2mm on *B. cereus*, while the methanol extract had value of 12±0.5mm. Hexane and DCM extracts had values of 14.6±0.2mm and 14.3±0.4mm repectively, on *S. aureus*, whereas methanol extract had the value of 9.6±0.2mm. From the results, all extracts of *A. altilis* were inactive on *P. aeruginosa.* Methanol extract had highest value of 9.5±0.2mm against *E. coli.* Antifungal activity for hexane showed the highest zone of inhibition of 11.8±0.3mm on *C. albicans,* followed by DCM extract 11.5±0.2mm, while the lowest value was methanol extract 7.5±0.2mm. Results on *C. neoformans*, hexane extract had the highest value of 9.6±0.2mm and DCM with 7.8±0.3mm zone of inhibition. The concentrations that used to evaluate the MIC for *A. altilis* were varied from (250 to 1000µg/ml). Tables 2 and 3 showed the results for MIC and MBC/MFC. The lowest MIC value for *A. altilis* extracts was at concentration of 250µg/ml obtained by hexane and methanol extracts on *S. aureus.* Similar MIC value of 250µg/ml was also obtained by hexane only on *C. albicans* and *C. neoformans* respectively. The highest MIC value was at 500µg/ml obtained by hexane, DCM on *B. cereus* and *E. coli.* Highest MBC value was obtained by DCM at 1000µg/ml on *E. coli.* The lowest MIC value for fungi strains was 250µg/ml obtained by hexane extract on *C. albicans* and *C. neoformans,* while the highest was DCM extract at 1000µg/ml, wherase MFC value was obtained by DCM at 1000µg/ml on *C. neoformans.* All the three extracts of *A. altilis* were active against the pathogenic microorganisms.

Discussion

The present study on *in vitro* antimicrobial activities of *A. altilis* (twigs part). The hexane, DCM and methanol of *A.altilis* (twigs) with concentration of (2mg/disc) were screened for antimicrobial activity against different strains ofbacteria and fungi which were known as pathogenic microorganisms. Generally the value of inhibition zones from 0-10mm are considered that sample has a weak antimicrobial activity, from 10-15mm are moderate and from 15 and above are strong activities (Najihah et al., 2012). The antimicrobial effects of the crude extracts of *A. altilis* (twigs) (Table 1) obtained using hexane showed the highest activity value of 14.6±0.2mm on *S. aureus* and *B.cereus,* while DCM and methanol extracts had slightly different value of 14±0.5mm against *B.cereus*. De Boer et al., (2005) suggested that the bioactive compounds might be soluble in non-polar and semi-polar organic solvents. From this result *A. altilis* extracts have great effects on *S. aureus* and *B. cereus* (Gram-positive) strains. Highest value (9.5±0.2mm) of methanolic extract of *A. altilis* on *E. coli* (Gram-negative) was recorded. The three crude extracts of *A. altilis* were inactive against *P. aeruginosa* and the resistance of *P. aeruginosa* due to influx of protein systemswhich are common in most pathogenic bacteria and are responsible for the removal of antibiotics and other toxins from bacterial cells, ensuring their survival (Banerjee et al., 2000; Borges-Walmsley & Walmsley, 2001). Hexane and DCM extracts of *A. altilis* were active on all fungal strains tested in this study, while only methanol extract was unable to inhibit the growth on *C. neoformans.* The variation in the effectiveness of the extract against different microorganisms depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. It has been suggested that the antimicrobial activity is mainly due to the presence of essential oils, flavanoids and triterpenoids and other natural polyphenolic compounds or free hydroxyl groups (Rojas et al., 2006). Many plants have been used because of their antimicrobial traits and the antimicrobial properties of plants have been investigated by a number of researchers worldwide.

Traditional healers use primarily water and plant extracts different from organic solvents have been found to give more consistent antimicrobial activity compared to the former (Parekh et al., 2005). External plant surfaces are often protected by biopolymers for example, waxes of fatty acid esters such as cutin and suberin. In addition, external tissues can be rich in phenolic compounds, alkaloids, diterpenoids, steroid alkaloids and other compounds which inhibit the development of fungi and bacteria. Cell walls of at least some monocotyledons also contain antimicrobial proteins, referred to as thionins (Angeh, 2006). The gram positive bacteria were found to be more sensitive to the extracts of *A. altilis* than the gram negative and fungul strains tested. The antibacterial activity was more active on the gram-positive bacteria (*S.aureus* and *B.cerues*) than the gram-negative bacteria (*E. coli* and *P. aeruginosa*) and

fungi (*C. albicans* and *C. neoformans*) respectively. This might be due to the differences in cell membrane constituents and arrangement (Negi et al., 2008), Gram-positive bacteria having an outer phospholipidic membrane carrying the structure lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand, are more sensitive having only an outer peptidoglycan layer which is not an effective force of barrier. Therefore, the cell walls of Gram negative organisms are more complex in layout than the Gram positive on acting as diffusional barrier and making them less susceptible to the antimicrobial agents than Gram positive bacteria (Alonso et al., 2005). The MIC value for the extracts of *A.altilis* twigs (Table 2) revealed that the hexane, DCM and methanol extracts had the least value of 250µg/ml againstGram positive bacteria strains (*S.aureus* and *B.cerues*). The MBC/MFC in this study also showed that *A. altilis* extracts gave the lowest value of 250µg/ml for Gram positive strains and fungal strains. Juliana (2004) reported that if the MIC and MBC/MFC ratio was found to be less than or equal to 5, the bacteria was considered to be effective. On the other hand, if this ratio was greater than 5, it was considered weak, and this in line with El-Mahmood & Doughari, (2008) reported that high MIC and MBC values are indication of low activity while low MIC and MBC are indication of high activity. In this study, *E. coli* had higher MIC and MBC values, thus suggesting lower efficacy of the crude extracts on (Gram negative) bacteria, while the extracts for both plants had lower values for *S. aureus* thus suggesting higher activity against the corresponding organism. From these results, MIC/MBC ratios of hexan, DCM and methanol extracts of *A. altilis* on Gram-positive bacteria were less than 5 and equal to 5 found out that twig extracts were active against some selected bacteria pathogens. Nweze et al., (2004) in line with Owoyale et al., (2005) reported that the antimicrobial activity against pathogens microorganisms of plants is associated with the presence of phytochemical components such as phenols, terpenoids, tannins, flavonoids, steroids and carbohydrates. The findings in this study support this, whereby antimicrobial activities of *A. altilis* extracts could be attributed to the presence of various secondary metabolites in different solvents. These observations support the usefulness of *A.altilis* in folklore remedies for the treatment of various illnesses and infections. in all the extracts of *A. altilis* have aremarkable antimicrobial activity growth inhibition effect achieved by interfering with division of microbial cell (Shahid & Mohammad, 2009) especially on the gram- positive bacteria but less effective on fungus. The *A. altilis* extracts were found to be active on at least one of the selected microbial strains. The antimicrobial activity of *A.altilis* against tested strains indicated that *S. aureus* was the most susceptible bacterium and *P. aeruginosa* was theleast susceptible on all microbial strains used in this study.

Conclusion

The research on in vitro antimicrobial investigation has determined for the first time. The results of the evaluation of the antimicrobial efficiency and MIC, MBC/MFC assay, revealed that all the three extracts of *A. altilis* demonstrated broad spectrum activities against the tested bacteria and fungi strains. The inhibition activities of the plant against pathogens microorganisms show enhanced disease resistance. More clinical and pharmacological studies shall be undertaken to establish the dose pattern. All types of plants including the present study were found to have activity at least on one of the selected microbial strains. These observations support the usefulness of *A. altilis* in folklore remedies for the treatment of various illnesses and infectious diseases especially those caused by the tested pathognic bacteria and fungi strains.

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Table1. Preliminary antimicrobial activity of *A. altilis* extracts (2mg/disc) zone of inhibition diameters in mm ± SD

A. altilis	**Microorganisms**					
Extracts	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*C. neoformans*
Hexane	14.6±0.2	14.6±0.2	9±0.5	Na	11.8±0.3	9.6±0.2
DCM	14.3±0.4	14.6±0.2	9±0.5	Na	11.5±0.2	7.8±0.3
Methanol	9.6±0.2	12±0.5	9.5±0.2	Na	7.5±0.2	Na
Positive control Streptomycin 10µg/disc and Nystatin 100 µg/disc	25±0.2	20.3±0.5	16.7±0.5	15.0±1	18.5±0.3	19.6±0.43
Negative control Methanol 30µg/disc	Na	Na	Na	Na	Na	Na

S.a = Staphylococcus aureus, E.coli = Escherichia coli, *B.c=Bacillus cereus, Ps.a= Pseudomonas aeruginosa, C.a= Candida albicans, C.n= Cryptococcus neoformans, SD= Standard Deviation n=3, Na =not active*

Table 2. Minimum inhibitory concentration (MIC) of *A. altilis* extracts on the bacterial and fungal strains

MIC (µg/ml)			
	Hexane	**DCM**	**Methanol**
Gram Positive			
S. aureus	250	500	250
B. cereus	500	500	250
Gram Negative			
E. coli	500	500	500
P. aeruginosa	-	-	-
Fungi			
C. albicans	250	1000	500
C. neoformans	250	1000	500

Table 3. Minimum bactericidal/fungicidal concentration of *A. altilis* extracts on bacterial and fungal strains

MBC/MFC (µg/ml)			
	Hexane	**DCM**	**Methanol**
Gram Positive			
S. aureus	500	500	500
B. cereus	500	1000	500
Gram Negative			
E. coli	500	1000	500
P. aeruginosa	-	-	-
Fungi			
C. albicans	500	500	500
C. neoformans	500	1000	500